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Application News

Ultra Fast Preparative and Purification LC System

# Automated Preparative Purification Using Nexera™ UFPLC

## —High Concentration Collection via an Online Trap Column—

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### User Benefits

- By repeatedly loading fractions onto the trap column, high-concentration collection is achieved.
- Because the collected fraction is organic solvent–rich, post-purification drying and powdering can be shortened.
- The target compound can be collected at high concentration regardless of peak shape.

## ■ Introduction

Preparative LC is widely used across the pharmaceutical, food, and chemical industries for applications such as purification of target compounds from mixtures, screening of active components in natural products, and structural elucidation of impurities and unknown compounds. In Application News 01-00937, a single analytical / preparative convertible LC-MS system was introduced to streamline the preparative workflow, including optimization of separation conditions at the analytical scale, scale-up and fractionation, and confirmation of purity and recovery. However, because target compounds are collected in a state diluted with mobile phase, the subsequent drying and powdering steps remain time-consuming. The ultra fast preparative and purification LC system "Nexera UFPLC" (Fig. 1) is a proprietary system equipped with functions that automate processes associated with preparative purification, including concentration and purification in addition to fractionation. This article presents a case in which Nexera UFPLC enables concentration of the target compound on an online trap column and its collection at high concentration in an organic solvent. This approach is expected to significantly reduce the effort required for post-purification drying and powdering steps.

*1 UFPLC：Ultra Fast Preparative and Purification Liquid Chromatograph

Fig. 1 Ultra Fast Preparative and Purification LC System "Nexera™ UFPLC"

## ■ Overview of Nexera UFPLC

Nexera UFPLC combines preparative LC with a trap column to automate processes such as concentration and purification in addition to fractionation. The details of each step are described below.

(1) The target compound in the mixture is separated by preparative LC and introduced into the trap column (fractionation / concentration).

(2) An appropriate solvent is passed through the trap column to remove counterions and nonvolatile salts (purification).

(3) An organic solvent is passed through the trap column to elute the target compound (elution).

A schematic overview of each step is shown in Fig. 2, and a simplified flow diagram of Nexera UFPLC is shown in Fig. 3.

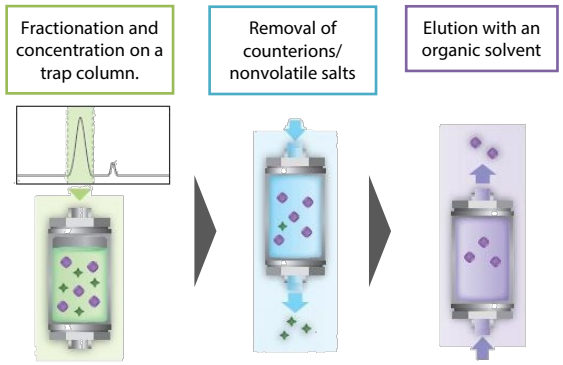


Fig. 2 Schematic Overview of Nexera UFPLC

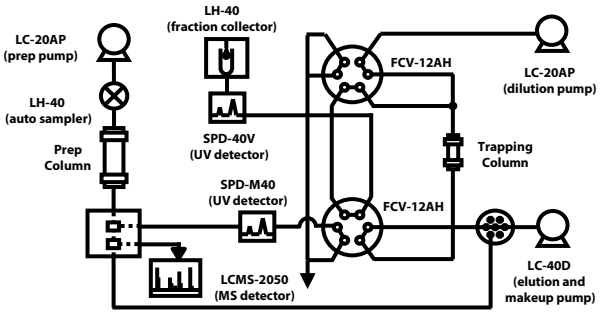


Fig. 3 Simplified Flow Diagram of Nexera UFPLC

## ■ Dedicated Software for Nexera UFPLC "Purification Solution™"

Nexera UFPLC is controlled using the dedicated software "Purification Solution" (Fig. 4). Because the chromatogram during fractionation, the trap column to which the collected peaks are introduced, and the elution chromatogram after purification are displayed on a single screen, the collection of the target compound can easily be monitored.

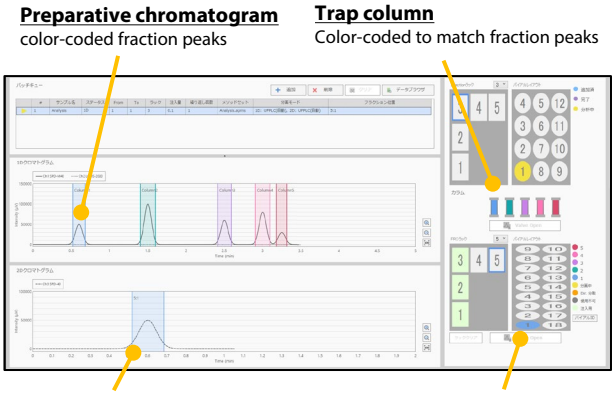


Fig. 4 Purification Solution Interface

In the following, a mixture of seven small molecule drugs is used as a model compound to demonstrate automated fractionation, concentration, and elution in an organic solvent of the target compound (Hydrocortisone) using Nexera UFPLC.

## ■ Analytical Conditions

The analytical conditions are shown in Table 1. The model sample was prepared with Hydrocortisone at 10 mg/mL and the other compounds at 1 mg/mL. Concentration on the trap column and collection with acetonitrile were automated for this sample.

Table 1 Analytical Conditions

| | |
|---|---|
| System | : Nexera UFPLC |
| Mobile Phase | : Pump A : 0.1% formic acid in water |
| | : Pump B : Acetonitrile |
| Column | : Shim-pack Scepter C18-120 |
| | (150 mm × 20 mm I.D., 5 µm)*1 |
| Trap Column | : Shim-pack UFPLC 20×30 |
| | (30 mm × 20 mm I.D., 20–30 µm)*2 |
| Sample | : (A) Hydrocortisone, (B) Salicylic acid, |
| | (C) Metoclopramide, (D) Lidocaine, |
| | (E) Furosemide, (F) Papaverine, (G) Quinidine |
| Sample Concentration | : 10 mg/mL (Hydrocortisone), |
| | 1 mg/mL (others) |
| Sample Solvent | : Water/Acetonitrile = 1:1 |
| Injection Volume | : 1 mL |
| **LC Conditions** | |
| Time Program | : B Conc. 15%(0 min)→45%(20 min) |
| | →15%(20.01-25 min) |
| Column Temp. | : Ambient |
| Flow rate (Prep) | : 20 mL/min |
| Flow rate (Dilution) | : 80 mL/min (Water) |
| Flow rate (Makeup) | : 1.5 mL/min (Methanol) |
| Flow rate (Elution) | : 9 mL/min (Acetonitrile) |
| Sample loop size | : 2 mL |
| Syringe size | : 5 mL |
| Detection (Prep) | : 245 nm (SPD-M40, high pressure preparative cell) |
| Detection (Elution) | : 245 nm (SPD-40, high pressure preparative cell) |
| **MS conditions** | |
| Ionization | : ESI/APCI (DUIS), positive |
| Mode | : SCAN (*m/z* 100–500), SIM |
| Nebulizing Gas Flow | : 2.0 L/min ($N_2$) |
| Drying Gas Flow | : 5.0 L/min ($N_2$) |
| Heating Gas Flow | : 7.0 L/min ($N_2$) |
| DL Temp. | : 200 ˚C |
| Desolvation Temp. | : 100 ˚C |
| Interface Voltage | : 3.0 kV |

*1 P/N : 227-31102-03 *2 P/N : 228-80220-41

## ■ Automated Preparative Purification Using Nexera UFPLC

The chromatogram and flow diagram (during fractionation / concentration) for the mixture of seven standard compounds are shown in Fig. 5. Hydrocortisone is separated on the preparative column and introduced into the trap column (flow path in red). At this stage, ultrapure water is simultaneously delivered from the dilution pump (flow path in blue) to ensure adequate retention and concentration of the target compound on the trap column. Nexera UFPLC is configured to split the preparative flow path and direct part of the column eluent to an MS (LCMS-2050) using a make-up pump (flow path in green), enabling the use of both UV and MS signals as triggers during fractionation. The elution chromatogram from the trap column and the corresponding flow diagram (during elution) are shown in Fig. 6. By delivering acetonitrile from the elution pump (flow path in green), Hydrocortisone concentrated on the trap column is recovered in an organic solvent–rich state. Furthermore, Nexera UFPLC enables repeated injections to continuously introduce the target compound onto the trap column. Fig. 7 shows the chromatogram obtained after introducing Hydrocortisone onto the trap column three times, followed by elution. Repeated injections allow the target compound to be recovered at higher concentration.

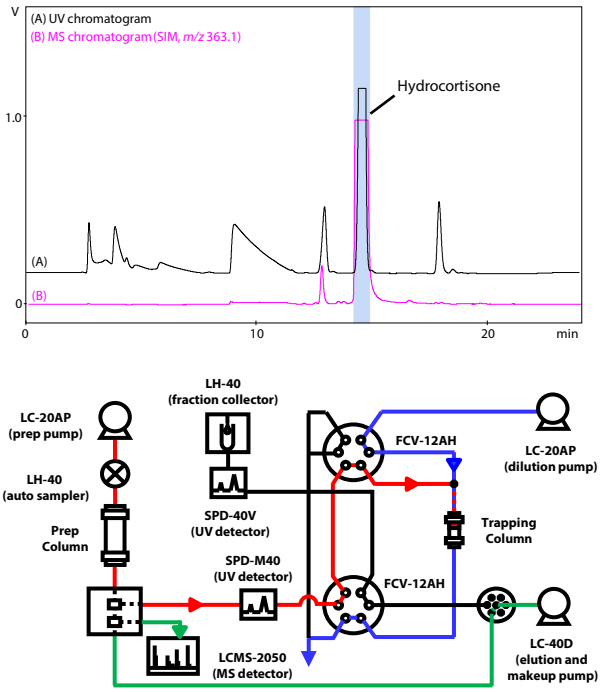


Fig. 5 Preparative Chromatograms of Standard Mixture (top)
Flow Diagram During Fractionation / Concentration (bottom)
*Blue area in the chromatogram indicates fraction introduced into the trap column

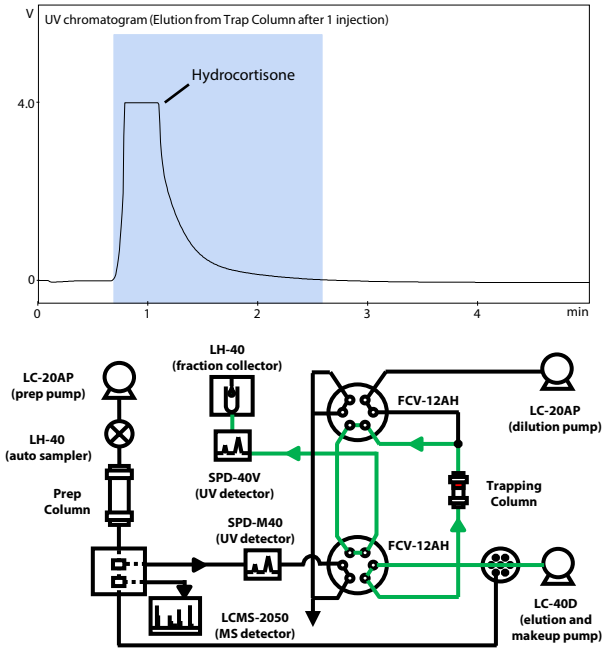


Fig. 6 Elution Chromatogram from Trap Column (top)
Flow Diagram During Elution (bottom)
*Blue area in the chromatogram indicates fractionation interval

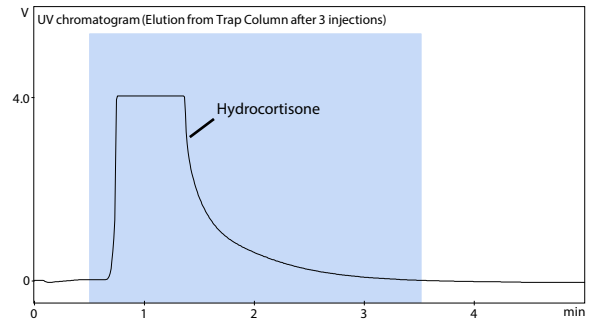


Fig. 7 Elution Chromatogram from Trap Column
*Eluted after three injections and loading
*Blue area in the chromatogram indicates fractionation interval

When post-preparative processes such as drying and powdering of collected fractions are required, conventional reversed-phase preparative LC is time-consuming because the target compound is collected in a state diluted with water in the mobile phase. In contrast, Nexera UFPLC enables high-concentration recovery of the target compound in an organic solvent, significantly reducing the effort required for post-preparative processes. Table 2 compares the collected fractions obtained by preparative LC and Nexera UFPLC using Hydrocortisone as the target compound. Chromatograms obtained by preparative LC and Nexera UFPLC are shown in Fig. 8 and Fig. 9, respectively. In preparative LC, the fractionation time was 1 min (flow rate: 20 mL/min), and the concentration of the collected fraction was 500 mg/L. In contrast, Nexera UFPLC showed a fractionation time of 3 min (flow rate: 9 mL/min) and a collected fraction concentration of 1111 mg/L, corresponding to approximately 2.2-fold higher concentration. Furthermore, whereas the solvent of the collected fraction in preparative LC was 36% acetonitrile in water, Nexera UFPLC enabled recovery in 100% acetonitrile.

Table. 2 Comparison of Collected Fractions Between Preparative LC and Nexera UFPLC

| | Conc. (mg/L) | Volume (mL) | Solvent |
|---|---|---|---|
| Prep LC | 500 | 20 | 36% ACN in water |
| UFPLC | 1111 | 27 | 100% ACN |

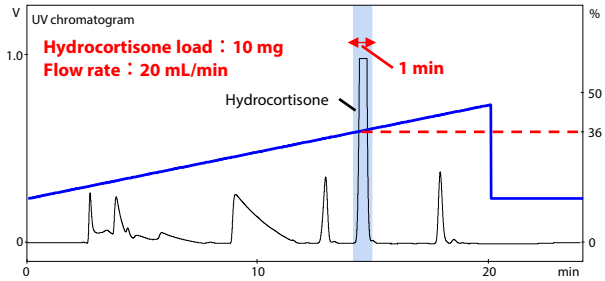


Fig. 8 Fractionation of Hydrocortisone by Preparative LC
*Blue line indicates the gradient conditions
*Blue area in the chromatogram indicates fractionation interval

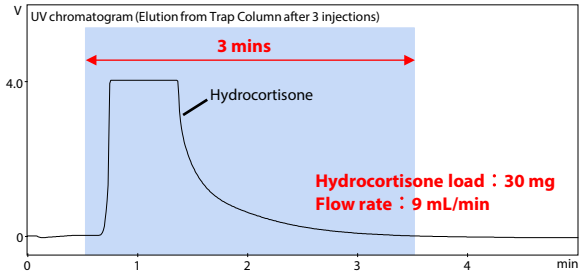


Fig. 9 Fractionation of Hydrocortisone by Nexera UFPLC
*Eluted after three injections and loading
*Blue area in the chromatogram indicates fractionation interval

## ■ Confirmation of Purity / Recovery

The chromatogram obtained by injecting Hydrocortisone collected using Nexera UFPLC into the analytical flow path (analytical conditions: Table 3), along with the chromatogram of a standard mixture prepared at the same concentration as the collected Hydrocortisone (reference for recovery calculation), is shown in Fig. 10. The purity and recovery are summarized in Table 4. Favorable results were obtained for both purity and recovery.

Table 3 Analytical Conditions

| | |
|---|---|
| System | : Nexera X3 |
| Mobile Phase | : Pump A : 0.1% formic acid in water |
| | : Pump B : Acetonitrile |
| Column | : Shim-pack Scepter C18-120 (150 mm × 4.6 mm I.D., 5 µm)*1 |
| Injection Volume | : 5 µL |
| Time Program | : B Conc. 15%(0 min)→45%(20 min) →15%(20.01-25 min) |
| Column Temp. | : Ambient |
| Flow rate | : 1 mL/min |
| Detection (PDA) | : 245 nm (SPD-M40, STD cell) |

*1 P/N : 227-31020-05

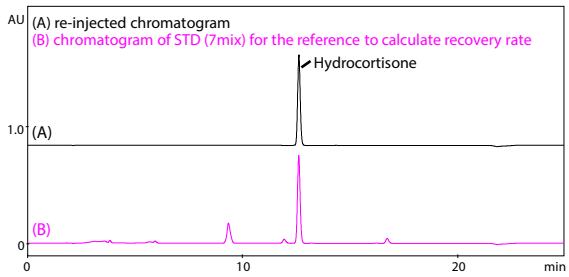


Fig. 10 Re-injected Chromatogram (A)
Chromatogram of STD (B)

Table 4 Purity and Recovery Rate of Fractionated Hydrocortisone

| | Purity (Area %) | Recovery Rate (%) |
|---|---|---|
| Hydrocortisone | 100.0 | 100.7 |

## ■ High Concentration Recovery Independent of Peak Shape

To achieve high-concentration recovery of the target compound, it is important to maintain sharp peak shapes and minimize the fractionation time. However, in some cases, mobile phase and column conditions that intentionally allow tailing of the target compound are selected to prioritize separation from closely eluting impurities. In addition, peak tailing may also occur as a result of increased sample loading. In such cases, conventional preparative LC may require longer fractionation times, leading to a decrease in the concentration of collected fractions. In contrast, Nexera UFPLC enables high-concentration recovery independent of the peak shape of the target compound, as the compound is concentrated on a trap column prior to collection. Table 5 compares the collected fractions obtained using Papaverine as the target compound for preparative LC and Nexera UFPLC. The corresponding chromatograms are shown in Fig. 11 and Fig. 12, respectively. In preparative LC, the fractionation time was 3 min (flow rate: 20 mL/min), and the concentration of the collected fraction was 17 mg/L. In contrast, with Nexera UFPLC, Papaverine was concentrated on the trap column, resulting in a fractionation time of 1 min (flow rate: 9 mL/min) and approximately 6.5-fold higher concentration (111 mg/L).

Table. 5 Comparison of Collected Fractions Between Preparative LC and Nexera UFPLC

| | Conc. (mg/L) | Volume (mL) | Solvent |
|---|---|---|---|
| Prep LC | 17 | 60 | 30% ACN in water |
| UFPLC | 111 | 9 | 100% ACN |

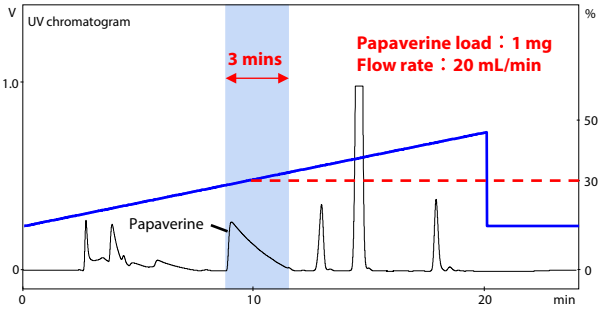


Fig. 11 Fractionation of Papaverine by Preparative LC
*Blue line indicates the gradient conditions
*Blue area in the chromatogram indicates fractionation interval

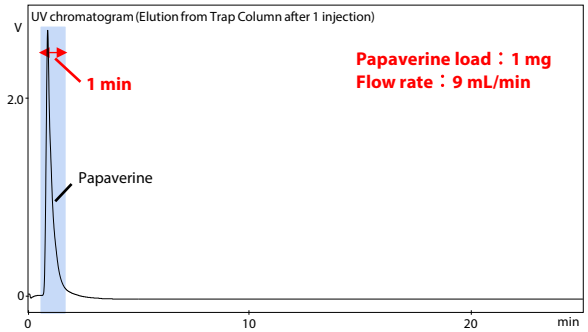


Fig. 12 Fractionation of Papaverine by Nexera UFPLC
*Blue area in the chromatogram indicates fractionation interval

## ■ Conclusion

In conventional reversed-phase preparative LC, the target compound is collected in a state diluted with water in the mobile phase, which makes post-preparative powdering a time-consuming process. Nexera UFPLC automates the concentration of the target compound and its collection in an organic solvent by utilizing a trap column. In addition, repeated sample injections enable the target compound to be introduced onto the same trap column, allowing higher-concentration recovery. This significantly reduces the effort required for post-preparative drying and powdering processes.



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